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# Prenatal exposure to per- and polyfluoroalkyl substances in association with autism spectrum disorder in the MARBLES study

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ABSTRACT

*Background:* Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) has shown potential to adversely affect child brain development, but epidemiologic evidence remains inconsistent. We examined whether prenatal exposure to PFAS was associated with increased risk of autism spectrum disorder (ASD).

*Methods:* Participants were 173 mother–child pairs from MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns), a high-risk ASD cohort. At 3 years old, children were clinically confirmed for ASD and classified into ASD ($n$ = 57) and typical development (TD, $n$ = 116). We quantified nine PFAS in maternal serum collected during pregnancy. We examined associations of ASD with individual PFAS as well as the combined effect of PFAS on ASD using scores of the first principal component (PC-1) accounting for the largest variance.

*Results:* Prenatal perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) showed positive associations (per 2 nanogram per milliliter increase: relative risk (RR) = 1.20, 95% CI: 0.90, 1.61 [PFOA]; RR = 1.24, 95% CI: 0.91, 1.69 [PFNA]), while perfluorohexane sulfonate (PFHxS) showed a negative association (RR = 0.88, 95% CI: 0.77, 1.01) with ASD risk. When examining associations of ASD with untransformed PFAS concentrations, PFOA, PFNA, and PC-1 were associated with increased ASD risk (per nanogram per milliliter increase: RR = 1.31, 95% CI: 1.04, 1.65; RR = 1.79, 95% CI: 1.13, 2.85; RR = 1.10, 95% CI: 0.97, 1.25, respectively), while the RR of PFHxS moved toward the null.

*Conclusions:* From this high-risk ASD cohort, we observed increased risk of ASD in children exposed to PFOA and PFNA. Further studies should be conducted in the general population because this population may have a larger fraction of cases resulting from genetic sources.

## 1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made fluorinated chemicals (Olsen et al. 2017). Because of their water- and stain-resistant properties, PFAS have been widely used in the manufacture of consumer and industrial products, including cookware non-stick coatings, food contact materials, stain- and water-resistant fabric coatings, industrial surfactants, and fire-fighting foams (Benford et al.

*Abbreviations:* ADHD, attention-deficit/hyperactivity disorder; ADOS, Autism Diagnostic Observation Schedules; ASD, autism spectrum disorder; BMI, body mass index; CDC, Centers for Disease Control and Prevention; CI, confidence interval; CSS, calibrated severity scores; DAG, directed acyclic graph; DSM-5, Diagnostic and Statistical Manual of Mental Disorders 5th Edition; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; LOD, limit of detection; ln, natural logarithm; MARBLES, Markers of Autism Risk in Babies – Learning Early Signs; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; MSEL, Mullen Scales of Early Learning; NHANES, National Health and Nutrition Examination Survey; Non-TD, non-typical development; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoate; PFDoDA, perfluorododecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoate; RR, relative risk; SD, standard deviation; TD, typical development.

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2008). Due to their wide range of applications and persistence in the environment, several PFAS have been frequently detected in the serum of the general U.S. population for the last 20 years (CDC 2019; Kato et al. 2011a).

Exposure to PFAS during pregnancy is of concern. PFAS can cross placenta and be transported to the fetus (Gützkow et al. 2012), and a number of PFAS have been detected in cord blood samples (Fisher et al. 2016; Kato et al. 2014). PFAS may adversely affect child's brain development. In animal studies, mice that were prenatally exposed to perfluorooctane sulfonate (PFOS) exhibited delays in developmental landmarks and neuromotor maturation (Fuentes et al. 2007). Prenatal exposure to perfluorooctanoate (PFOA) resulted in alterations in exploratory behaviors in male and female mice (Onishchenko et al. 2011). In humans, there is evidence that PFAS disrupt thyroid hormone homeostasis of pregnant women (Berg et al. 2015; Wang et al. 2014), which may adversely affect fetal brain development (Morreale de Escobar et al. 2000). Several prospective birth cohort studies showed that maternal thyroid hormone deficiency during pregnancy is associated with increased risk of child neurodevelopmental delay, attention-deficit/hyperactivity disorder (ADHD) symptoms, and autistic behaviors in infancy and childhood (Modesto et al. 2015; Pop et al. 2003; Román et al. 2013). However, previous epidemiological findings on the associations between prenatal exposure to PFAS and the risk of neurodevelopmental concerns or autistic behaviors have been inconclusive (Liew et al. 2018).

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impairments in social communication and interaction, restricted interests and repetitive behaviors, and sensory sensitivities. A growing body of literature suggests that exposure to environmental neurotoxicants during pregnancy can contribute to the development of ASD (Hertz-Picciotto et al., 2018a). Five epidemiologic studies examined the associations between prenatal PFAS exposure and the risk of ASD. A prospective cohort study observed that higher PFOA, but not other PFAS, in 16-week prenatal maternal serum was associated with less autistic behaviors in 4- and 5-year-old children (Braun et al. 2014). A nested case-control study did not find convincing evidence of associations between maternal PFAS plasma concentrations collected during the first or second trimesters of pregnancy and increased risk of childhood autism (Liew et al. 2014). Two case-control studies reported that higher PFOA and PFOS in 15- to 19-week prenatal maternal serum (Lyall et al., 2018) or PFOS in amniotic fluid collected during the second trimester (Long et al., 2019) were associated with decreased risk of child ASD, while other PFAS were not associated. Another case-control study observed that modeled prenatal maternal serum PFOS and perfluorohexane sulfonate (PFHxS), but not other PFAS, were associated with increased risk of child ASD (Shin et al. 2020). Because PFAS are weakly or moderately correlated each other and one PFAS may confound another PFAS, consideration of a single compound in the model may explain, at least in part, the inconsistent findings from these studies.

In this study, we used maternal serum samples prospectively collected during pregnancy in a high-risk ASD cohort to examine whether prenatal maternal serum PFAS concentrations were associated with increased risk of child ASD. We also examined the potential combined effect of exposure to a mixture of PFAS on ASD risk.

## 2. Methods

### 2.1. Study population

MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns) is a prospective cohort study, which began in 2006, that enrolls women who are pregnant with a child who has a first degree relative with ASD and thereby is at elevated risk (~20%) for developing ASD (Hertz-Picciotto et al., 2018b; Ozonoff et al., 2011). Participants are primarily recruited from families receiving services for children with ASD through the California Department of Developmental Services. Inclusion criteria of MARBLES are: 1) mother or father has a child or other first degree relative with ASD; 2) mother is 18 years old or older; 3) mother is pregnant; 4) mother speaks, reads, and understands English; and 5) mother resides within 2.5 h of the Davis/Sacramento region at the time of enrollment. For families who consent to participate in the MARBLES study, demographic information, medical records, and biological specimens are prospectively collected. Detailed information on study design, study population, inclusion criteria, recruitment, and data collection is described elsewhere (Hertz-Picciotto et al., 2018b).

For the present study, we included 193 mothers who provided blood samples during pregnancy between 2009 and 2015. One mother who did not have a previous child with ASD at enrollment was included in this study because she had three siblings with ASD. Among 193 mothers, eight mothers participated in the study for two pregnancies and three mothers delivered twins. Thus, 204 mother-child pairs comprised the study population (Fig. 1).

### 2.2. Serum sample collection and PFAS quantification

A total of 312 maternal blood samples were collected during pregnancy from 193 mothers. Each mother provided a varying number of blood samples during pregnancy (mean = 1.5; standard deviation (SD) = 0.8), with most samples (79%) being provided in the second and third trimesters. Of 312 samples, 67 were collected in the first trimester, 142 in the second trimester, and 103 in the third trimester. Of 204 mother-child pairs, 136 pairs provided one sample, 28 pairs provided two samples, and 40 pairs provided three samples (Fig. 1). Whole blood was centrifuged, and separated serum was stored at −80 °C within 24 h of blood draw.

Quantification of PFAS in maternal sera was performed at the Centers for Disease Control and Prevention (CDC) using online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–isotope dilution tandem mass spectrometry. Detailed analytical methods for quantification of PFAS are described elsewhere (Kato et al., 2011b). For quality control, blank samples and two quality control materials (low and high concentrations) were included in each batch. For quality assurance, we additionally analyzed 25 blind duplicate samples along with the study samples. The median coefficient of variation for these duplicate pairs of samples varied from 0% to 11%, depending on the analyte.

Nine PFAS were quantified in serum: PFOA, PFOS, PFHxS, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA). The limit of detection (LOD) for all nine PFAS was 0.1 ng/mL. For PFAS concentrations below the LOD, we used instrument-observed values as there seems to be less bias with this approach than using an imputation method that assigns the same value (e.g., LOD/2) to all non-detected concentrations (Lubin et al., 2004; Richardson and Ciampi, 2003).

### 2.3. Child neurodevelopmental assessment

At approximately 36 months of age, children were administered the Autism Diagnostic Observation Schedule (ADOS), which is a semi-structured, standardized diagnostic assessment of ASD conducted by a trained psychologist (Ozonoff et al. 2005). Raw ADOS scores were further converted into calibrated severity scores (CSS) ranging from 1 to 10 (CSS $\geq$ 4 represents ASD classification) (Esler et al. 2015). Children were also assessed for cognitive development using Mullen Scales of Early Learning (MSEL) which has scores for four subscales (i.e., visual reception, fine motor, receptive language, and expressive language) (Mullen, 1997). Based on ADOS and MSEL scores, we defined the neurodevelopmental outcome of each child and classified 204 children into three diagnostic groups using a previously published algorithmic

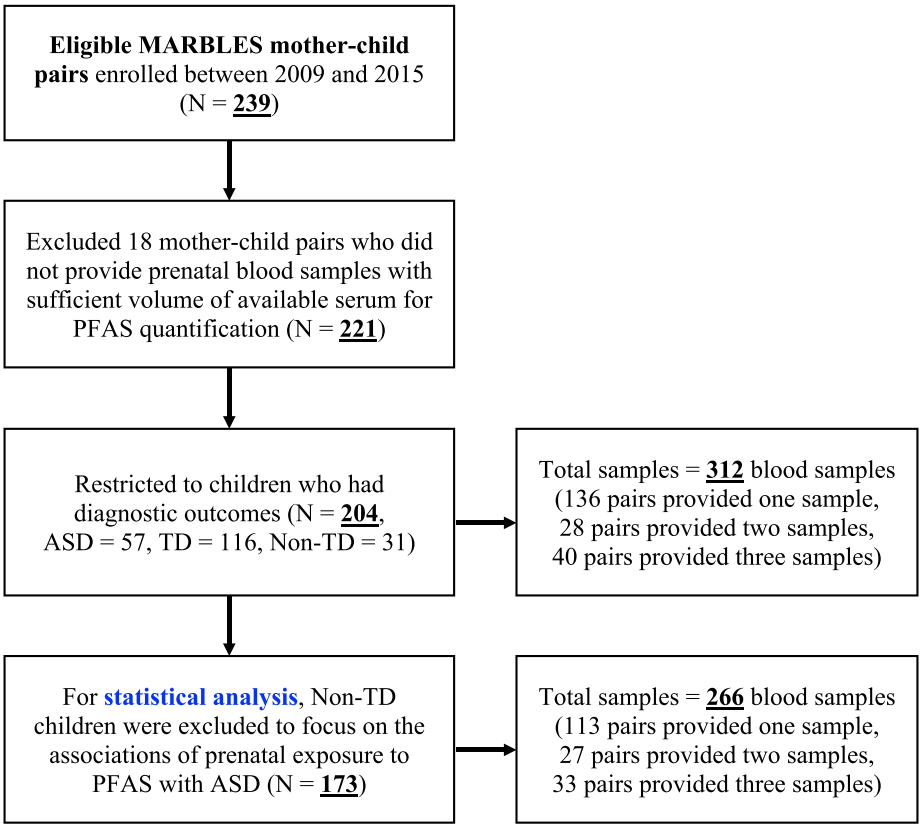


**Fig. 1.** Study flow and blood sample collection from MARBLES participants.

method with some modifications (Esler et al. 2015; Ozonoff et al. 2014). Children who met *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (DSM-5) criteria for ASD and had ADOS CSS $\geq$ 4 were classified into ASD ($n$ = 57). Children who did not meet DSM-5 criteria for ASD but had ADOS CSS $\geq$ 3 and/or lower MSEL scores (>1.5 SD below the mean in two or more MSEL subscales or > 2 SD below the mean in at least one MSEL subscales) were classified into non-typical development (Non-TD; $n$ = 31). The rest were classified into typically developing children (TD; $n$ = 116) (Hertz-Picciotto et al., 2018b). The children with Non-TD diagnosis were excluded from the current study, as our focus was on ASD specifically. Therefore, 173 mother-child pairs were used in the statistical analyses.

### 2.4. Statistical analysis

We computed univariate statistics and compared population characteristics of ASD and TD groups using the Pearson's chi-squared test for categorical variables and the Wilcoxon rank-sum test for a continuous variable. We compared median PFAS concentrations between the two diagnostic groups using the Wilcoxon rank-sum test. We also compared median PFAS concentrations among groups of population characteristics using the Wilcoxon rank-sum test for binary variables and the Kruskal-Wallis test for other categorical variables. Further, we compared our median PFAS concentrations with those of the pregnant women ($n$ = 61) reported in the National Health and Nutrition Examination Survey (NHANES) during the same study period (2009–2014). We combined PFAS concentration data from three NHANES cycles (i.e., 2009–2010, 2011–2012, 2013–2014) and computed median PFAS concentrations restricted to the pregnant women in the NHANES population (CDC, 2019).

We used Poisson regression models with robust error variance to examine the associations between prenatal PFAS concentrations and the relative risks (RRs) of ASD, compared with TD (Chen et al. 2018). We constructed a directed acyclic graph (DAG) including *a priori* chosen potential confounders and risk factors for ASD identified in the previous studies (Figure S1). We evaluated percent changes of the beta estimate by excluding each covariate from the DAG full model and selected only those covariates which changed the estimate by greater than 10% (Weng et al. 2009). In the final model, we included child's sex (female, male), birth year (2009–2010, 2011–2013, 2014–2015), maternal vitamin intake in the first month of pregnancy (yes, no), and two variables as proxies of socioeconomic status: maternal education (high school or some college credit, bachelor's degree, graduate or professional degree) and homeownership (owner, non-owner). For mothers who delivered twins or were enrolled for two children from different pregnancies, we adjusted for within-family correlations by participant identification numbers in the regression models by using clustered sandwich variance estimators (Zou 2004). Because there were missing values in two covariates, we performed multiple imputation by chained equations, which included all exposure and outcome variables as well as the selected covariates (White et al. 2011). Ten imputed datasets were generated and used in the regression models.

Using the final Poisson regression models, we estimated adjusted RRs and 95% confidence intervals (CIs) for four PFAS which were detected in more than 99% of the study samples (i.e., PFOA, PFOS, PFHxS, and PFNA). We fit two different models for each of the individual PFAS using (1) log 2-transformed (or binary log-transformed) PFAS concentrations, describing the two-fold increase in the log of the RR in relation to the PFAS concentrations (to lessen the influence of outlying measurements from the skewed untransformed distributions) and (2) PFAS concentrations without transformation, describing the linear increase in the log of the RR in relation to the PFAS concentrations (because a previous study suggested a linear dose–response relationship between the risk of ASD and PFAS concentrations) (Shin et al. 2020). For mothers who provided multiple samples during pregnancy, we used average PFAS concentrations in the regression models. In order to examine the

combined effect of the four PFAS concentrations on ASD, we performed a principal component analysis which reduces a set of correlated variables into a smaller number of principal components (PCs). We selected a PC that had eigenvalue greater than one based on the Kaiser's criterion (Kaiser 1960) and used its scores in the regression models.

As a sensitivity analysis, we excluded the top 2.5 percentiles of individual PFAS concentrations and ran the regression models to examine the effect of extreme values. We also ran the regression models by additionally adjusting for a set of potential confounders identified from a DAG: maternal pre-pregnancy body mass index (BMI) (normal/underweight, overweight, obese), parity (≤1, > 1), maternal age at delivery (<35 years, ≥ 35 years), and maternal race/ethnicity (non-Hispanic white, Hispanic, other). For mothers who provided more than one sample during pregnancy, we additionally ran the same final models using the highest concentration or a randomly selected concentration among multiple samples instead of their average. To evaluate effect modification of the associations between PFAS and ASD risk, we examined child's sex (female, male) and maternal age at delivery (<35 years, ≥ 35 years) as potential effect modifiers on the grounds of biological plausibility (Braun et al. 2014; Janecka et al. 2017; Jeddy et al. 2017; Werling and Geschwind 2013). We compared stratum-specific estimates and examined *p*-values for the interaction terms. Statistical analyses were performed using STATA/IC version 15.1 (StataCorp LLC, College Station, TX, USA).

## 3. Results

### 3.1. Population characteristics

In this study, we observed differences in several population characteristics between ASD and TD groups (Table 1). As expected, the male to female ratio of children with ASD (2.35) was higher than TD children (1.15). Mothers of TD children were more likely to have a bachelor's degree or higher (53.4%), compared to those of children with ASD (36.8%) and more likely to own a home (60.3%) than those of ASD children (45.6%). Fewer mothers who had children with ASD took prenatal vitamins during the first month of pregnancy (40.4%), compared to those who had TD children (56.0%).

### 3.2. Prenatal maternal PFAS serum concentrations and principal component

The detection frequency of PFOA, PFOS, PFHxS, and PFNA was 100%, 100%, 99%, and 99.6%, respectively, and five other PFAS were detected in less than 85% of the samples (Table 2). We observed the highest median for PFOS (3.0 ng/mL), followed by PFOA (0.9 ng/mL), PFNA (0.5 ng/mL), and PFHxS (0.4 ng/mL). The median PFOA of the ASD group was higher than that of the TD group (*p*-value = 0.02). There was no statistically significant difference in the medians of other PFAS between the two diagnostic groups. The medians of PFOA, PFOS, PFHxS, and PFNA in our study were slightly lower than those of pregnant women reported in NHANES, while those of other five PFAS concentrations were similar. At least one of the four PFAS detected in more than 99% of the samples (i.e., PFOA, PFOS, PFHxS, and PFNA) differed by child's sex and birth year, maternal age at delivery, maternal pre-pregnancy BMI, gestational age at delivery, homeownership, parity, and prenatal vitamin intake (Table S1).

Among 40 mothers who provided samples during all three trimesters, geometric mean of PFOA, PFOS, and PFNA was the highest in the first trimester and slightly decreased over time (Table S2). The correlation coefficients between the pairs of trimesters for PFOA, PFOS, and PFNA were greater than 0.85, indicating the stability of these three PFAS concentrations during pregnancy (Table S3). For the same four PFAS, concentrations were weakly or moderately correlated each other ($r$ = 0.36–0.65) (Table S4). From the principal component analysis, the first principal component (PC-1) was individually selected based on the log 2-transformed and untransformed PFAS, accounting for approximately 67% and 55% of the variance, respectively (Table S5). The two PC-1s had moderate positive loadings on all four PFAS (weight = 0.36 to 0.57). The scores for PC-1 between the two groups were not statistically different (*p*-value = 0.15 for log 2-transformed PFAS; *p*-value = 0.12 for untransformed PFAS).

**Table 1**
Characteristics of the study participants by diagnostic group.

| Characteristics [a] | All children ($n$ = 173) $n$ | ASD ($n$ = 57) $n$ | % | TD ($n$ = 116) $n$ | % | p-value [b] |
|---|---|---|---|---|---|---|
| **Child's sex** | | | | | | 0.04 |
| Female | 71 | 17 | 29.8 | 54 | 46.6 | |
| Male | 102 | 40 | 70.2 | 62 | 53.4 | |
| **Child's birth year** | | | | | | 0.53 |
| 2009–2010 | 61 | 19 | 33.3 | 42 | 36.2 | |
| 2011–2013 | 57 | 22 | 38.6 | 35 | 30.2 | |
| 2014–2015 | 55 | 16 | 28.1 | 39 | 33.6 | |
| **Gestational age at delivery** | | | | | | 0.37 |
| ≤ 37 weeks | 10 | 2 | 3.5 | 8 | 6.9 | |
| > 37 weeks | 163 | 55 | 96.5 | 108 | 93.1 | |
| **Maternal age at delivery** | | | | | | 0.91 |
| < 35 years | 93 | 31 | 54.4 | 62 | 53.4 | |
| ≥ 35 years | 80 | 26 | 45.6 | 54 | 46.6 | |
| **Maternal BMI at pre-pregnancy** | | | | | | 0.37 |
| Normal/underweight | 83 | 23 | 40.4 | 60 | 51.7 | |
| Overweight | 50 | 19 | 33.3 | 31 | 26.7 | |
| Obese | 40 | 15 | 26.3 | 25 | 21.6 | |
| **Gestational diabetes** | | | | | | 0.56 |
| Yes | 32 | 12 | 21.1 | 20 | 17.2 | |
| No | 140 | 45 | 78.9 | 95 | 81.9 | |
| **Maternal race/ethnicity** | | | | | | 0.95 |
| Non-Hispanic white | 94 | 30 | 52.6 | 64 | 55.2 | |
| Hispanic | 41 | 14 | 24.6 | 27 | 23.3 | |
| Other [c] | 38 | 13 | 22.8 | 25 | 21.6 | |
| **Maternal education** | | | | | | 0.09 |
| High school, some college | 90 | 36 | 63.2 | 54 | 46.6 | |
| Bachelor's degree | 50 | 11 | 19.3 | 39 | 33.6 | |
| Graduate or professional | 33 | 10 | 17.5 | 23 | 19.8 | |
| **Homeownership** | | | | | | 0.11 |
| Yes | 96 | 26 | 45.6 | 70 | 60.3 | |
| No | 75 | 29 | 50.9 | 46 | 39.7 | |
| **Parity** | | | | | | 0.52 |
| 0 | 2 | 0 | 0.0 | 2 | 1.7 | |
| 1 | 70 | 21 | 36.8 | 49 | 42.2 | |
| > 1 | 99 | 34 | 59.6 | 65 | 56.0 | |
| **Maternal vitamin intake in the first month of pregnancy** | | | | | | 0.07 |
| Yes | 88 | 23 | 40.4 | 65 | 56.0 | |
| No | 84 | 33 | 57.9 | 51 | 44.0 | |
| | | **Mean (SD)** | | **Mean (SD)** | | |
| **Breastfeeding duration (months)** | | 11.5 (10.3) | | 11.7 (9.5) | | 0.61 |

[a] Missing information ($n$): gestational diabetes (1), homeownership (2), parity (2), maternal vitamin intake in the first month of pregnancy (1), breastfeeding duration (10).
[b] P-value from the Pearson's chi-squared test for categorical variables and the Mann-Whitney test for continuous variables.
[c] Includes Black/African American (3%), Asian (16%), and multiracial (3%).

### 3.3. Associations between prenatal maternal PFAS serum concentrations and ASD risk

After adjusting for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy, using log 2-transformed exposure measures mostly produced null associations between ASD risk and maternal prenatal PFAS serum concentrations, including PC-1 scores (Table 3). PFOA and PFNA showed positive associations with increased ASD risk, though the

**Table 2**
Distribution of nine PFAS concentrations (ng/mL) in 266 maternal serum samples collected from 173 mother–child pairs.

| PFAS [a] | LOD (ng/mL) | % detect | All children ($n = 173$) | | | ASD ($n = 57$) | | | TD ($n = 116$) | | | p-value [a] | NHANES pregnant women median [b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Percentiles | | | Percentiles | | | Percentiles | | | | |
| | | | 5th | 50th | 95th | 5th | 50th | 95th | 5th | 50th | 95th | | |
| PFOA | 0.1 | 100 | 0.3 | 0.9 | 2.3 | 0.4 | 1.1 | 2.4 | 0.3 | 0.9 | 2.2 | 0.02 | 1.3 |
| PFOS | 0.1 | 100 | 1.1 | 3.0 | 6.8 | 1.0 | 3.2 | 7.0 | 1.2 | 2.9 | 6.7 | 0.23 | 3.7 |
| PFHxS | 0.1 | 99 | 0.2 | 0.4 | 1.6 | 0.2 | 0.4 | 1.7 | 0.2 | 0.4 | 1.5 | 0.45 | 0.6 |
| PFNA | 0.1 | 99.6 | 0.2 | 0.5 | 1.0 | 0.2 | 0.5 | 1.0 | 0.2 | 0.5 | 1.1 | 0.32 | 0.6 |
| PFDA | 0.1 | 83 | <LOD | 0.1 | 0.4 | <LOD | 0.1 | 0.4 | <LOD | 0.1 | 0.3 | 0.78 | 0.2 |
| PFUnDA | 0.1 | 60 | <LOD | 0.1 | 0.3 | <LOD | 0.1 | 0.4 | <LOD | 0.1 | 0.3 | 0.46 | <LOD |
| PFDoDA | 0.1 | 34 | <LOD | <LOD | 0.1 | <LOD | <LOD | 0.1 | <LOD | <LOD | 0.1 | 0.64 | <LOD |
| MeFOSAA | 0.1 | 57 | <LOD | 0.1 | 0.8 | <LOD | 0.1 | 1.1 | <LOD | 0.1 | 0.7 | 0.58 | 0.1 |
| EtFOSAA | 0.1 | 4 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.46 | <LOD |

Abbreviation: autism spectrum disorder (ASD), limit of detection (LOD), National Health and Nutrition Examination Survey (NHANES), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDA), 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), typical development (TD).

[a] P-value from the Wilcoxon rank-sum test comparing ASD and TD groups.

[b] Median PFAS concentrations of pregnant women from 2009 to 2010, 2011–2012, 2013–2014 NHANES ($n = 61$).

**Table 3**
Adjusted relative risk (RR) and 95% confidence interval (CI) for ASD ($n = 57$) versus TD ($n = 116$) in association with prenatal maternal PFAS concentrations and principal component scores.

| PFAS (ng/mL) [a] or principal component score | Log 2-transformed PFAS RR [b] (95% CI) | Untransformed PFAS RR [b] (95% CI) |
|---|---|---|
| PFOA | 1.20 (0.90, 1.61) | 1.31 (1.04, 1.65) |
| PFOS | 0.97 (0.74, 1.28) | 0.99 (0.90, 1.08) |
| PFHxS | 0.88 (0.77, 1.01) | 0.90 (0.54, 1.51) |
| PFNA | 1.24 (0.91, 1.69) | 1.79 (1.13, 2.85) |
| PC-1 [c] | 1.03 (0.87, 1.22) | 1.10 (0.97, 1.25) |

[a] Four PFAS detected in more than 99% of the study samples were individually included in the Poisson regression model.

[b] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[c] Selected from the principal component analysis because its eigenvalue was higher than 1.

associations were slightly above the null (per 2 nanogram per millimeter increase: RR = 1.20, 95% CI: 0.90, 1.61 for PFOA; RR = 1.24, 95% CI: 0.91, 1.69 for PFNA), while PFHxS showed a negative association with increased ASD risk (RR = 0.88, 95% CI: 0.77, 1.01). When using PFAS concentrations without transformation, RRs for PFOA, PFOS, and PC-1 increased with narrower CIs. ASD risk was increased with higher PFOA (per nanogram per millimeter increase: RR = 1.31, 95% CI: 1.04, 1.65), PFNA (RR = 1.79, 95% CI: 1.13, 2.85), and PC-1 (RR = 1.10, 95% CI: 0.97, 1.25), while the association of ASD risk with PFHxS moved toward the null (RR = 0.97, 95% CI: 0.54, 1.51).

When excluding the top 2.5 percentiles of individual PFAS concentrations, results of the models with log 2-transformed PFAS were similar, while the models with untransformed PFAS showed broader CIs (Table S6). When additionally adjusting for potential confounders (i.e., maternal pre-pregnancy BMI, parity, maternal age at delivery, and maternal race/ethnicity) or when using the highest concentration or a randomly selected PFAS concentration instead of the average concentration, similar results were observed in models with concentrations for both log 2-transformed PFAS and untransformed PFAS concentrations (Table S7).

**Table 4**
Adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (versus TD) in association with prenatal maternal PFAS concentrations, with log 2-transformation, stratified by child's sex and maternal age at delivery.

| Log 2-transformed PFAS (ng/mL) or principal component | RR (95% CI) | | p-value [a] |
|---|---|---|---|
| *Child's sex* [b] | Females [c] | Males [d] | |
| PFOA | 1.20 (0.79, 1.80) | 1.25 (0.87, 1.81) | 0.91 |
| PFOS | 0.99 (0.64, 1.54) | 1.07 (0.77, 1.48) | 0.86 |
| PFHxS | 0.80 (0.68, 0.95) | 1.04 (0.81, 1.33) | 0.31 |
| PFNA | 1.25 (0.76, 2.06) | 1.20 (0.82, 1.76) | 0.66 |
| PC-1 | 1.00 (0.77, 1.31) | 1.10 (0.90, 1.35) | 0.80 |
| *Maternal age at delivery* [e] | < 35 years [f] | ≥ 35 years [g] | |
| PFOA | 0.91 (0.61, 1.35) | 2.29 (1.30, 4.04) | 0.04 |
| PFOS | 0.65 (0.44, 0.98) | 1.67 (1.03, 2.71) | 0.02 |
| PFHxS | 0.72 (0.59, 0.89) | 1.30 (0.90, 1.89) | 0.08 |
| PFNA | 0.90 (0.58, 1.39) | 2.16 (1.23, 3.78) | 0.05 |
| PC-1 | 0.83 (0.68, 1.01) | 1.61 (1.16, 2.23) | 0.01 |

[a] P-value for interaction term of PFAS with each potential effect modifier (potential effect modifiers and an interaction term were added as additional terms in the regression model, along with other covariates).

[b] Adjusted for child's birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[c] Females ($n = 71$; TD = 54, ASD = 17), [d] Males ($n = 102$; TD = 62, ASD = 40).

[e] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[f] < 35 years ($n = 93$; TD = 62, ASD = 31), [g] ≥ 35 years ($n = 80$; TD = 54, ASD = 26).

When analyses were stratified by maternal age at delivery (<35, ≥ 35 years old), we observed effect modification of associations between ASD risk and all four PFAS concentrations as well as PC-1 scores (*p*-value for interaction ≤ 0.10) (Table 4). Among mothers who were 35 years old or older, ASD risk was increased with higher PFOA (RR = 2.29, 95% CI: 1.30, 4.04), PFOS (RR = 1.67, 95% CI: 1.03, 2.71), PFNA (RR = 2.16, 95% CI: 1.23, 3.78), and PC-1 (RR = 1.61, 95% CI: 1.16, 2.23). On the other hand, among mothers who were younger than 35 years old, ASD risk was decreased with higher PFOS (RR = 0.65, 95% CI: 0.44, 0.98), PFHxS (RR = 0.72, 95% CI: 0.59, 0.89), and PC-1 (RR = 0.83, 95% CI: 0.68, 1.01). When analyses were stratified by child's sex, we did not find sex-specific associations of any PFAS compounds with ASD risk (*p*-value for interaction > 0.30), and effect size estimates and 95% CI were broadly consistent across strata. When untransformed PFAS concentrations were used in the stratified analyses, the effect modification by maternal age at delivery were similarly found and sex-specific associations were not observed for any PFAS compounds (Table S8).

## 4. Discussion

In the present study, we examined whether prenatal exposure to PFAS was associated with increased risk of ASD using maternal serum samples collected during pregnancy in a high-risk ASD cohort. When using log 2-transformed PFAS concentrations, we found evidence of positive associations of ASD risk with prenatal PFOA and PFNA and inverse association with PFHxS. When the analyses were stratified by maternal age at delivery, each of the individual PFAS was associated with increased risk of ASD among mothers who were 35 years old or older and decreased risk of ASD among mothers who were younger than 35 years old. When untransformed PFAS concentrations were used in the models, we observed that higher PFOA, PFNA, and PC-1 (i.e., the first principal component with the largest variance from PCA) were associated with increased risk of ASD and the negative associations of PFHxS with ASD risk moved toward the null. The models with untransformed PFAS resulted in smaller standard errors of RRs for PFOA and PFOS compared to those with log 2-transformed PFAS. However, after excluding the extreme values (i.e., top 2.5 percentiles), the RRs and confidence intervals from the models with untransformed concentrations were relatively unstable compared to those from the models with log 2-transformed concentrations. Therefore, we cannot exclude the possibility that the models with untransformed concentrations may have been driven more by the influence of outliers and the results of the untransformed PFAS models should be interpreted with caution.

This current study did not show consistent results with previous studies that examined associations between prenatal PFAS exposure and the risk of ASD. Braun et al. observed that higher PFOA was associated with lower Social Responsive Scale scores, indicating fewer autistic behaviors in children, while PFOS, PFHxS, and PFNA showed null associations (Braun et al. 2014). They used continuous scales, rather than a clinical diagnosis, to assess autistic-like behaviors in a general population sample, limiting direct comparison of their results with our study. Liew et al. found evidence that higher PFHxS was associated with increased risk of childhood autism but other PFAS did not show apparent associations (Liew et al. 2014). Lyall et al. observed that most of the PFAS were not associated with the risk of ASD, with the exception that PFOA and PFOS showed associations with decreased ASD risk (Lyall et al. 2018). Compared to our study, these two studies (Liew et al. 2014; Lyall et al. 2018) had higher maternal plasma or serum concentrations of PFOA, PFOS, and PFHxS, while their PFNA concentrations were similar. Long et al. found that ASD risk was decreased with both higher PFOS and a principal component that was dominated by PFAS congeners in amniotic fluid (Long et al. 2019). However, their results may not be directly comparable to ours because PFOS was only detected in fewer than 50% of amniotic fluid samples and correlations between maternal serum and amniotic fluid concentrations varied considerably by PFAS (Stein et al. 2012). In a case-control study, Shin et al. used modeled prenatal maternal PFAS concentrations from maternal samples collected when their child was 2–5 years old and separately fit three different models using ln-transformed PFAS concentrations, PFAS concentrations with no transformation, and categorized PFAS concentrations (Shin et al. 2020). They observed that prenatal ln-transformed PFHxS was associated with increased risk of ASD, and additionally found that PFOS was borderline associated with the ASD risk when using PFAS concentrations without transformation.

Advanced maternal age (mostly considered to be 35 years old or older) was shown to contribute to child's neurodevelopmental disorders, including ASD (Janecka et al. 2017; Shelton et al. 2010). From the stratified analyses by maternal age at delivery, we observed that prenatal exposure to PFOA, PFOS, PFHxS, and PFNA as well as the combined PFAS was consistently associated with increased risk of having a child with ASD among mothers who delivered their child at 35 years old or older. A previous study also observed that prenatal maternal serum concentrations of PFOA and PFOS were associated with decreased communication development scores of 38-month-old females among mothers who were over 30 years old at delivery (Jeddy et al. 2017). One potential mechanism for the effect modification is age-induced epigenetic changes. During aging, epigenetic changes influenced by environmental exposures are accumulated and thus may lead to alterations in various gene expression pathways critical for fetal development, resulting in the development of ASD (Banik et al. 2017; Fraga and Esteller 2007). Changes in maternal hormone levels during pregnancy, which are altered by advanced maternal age, can also affect fetal brain development (Barrett et al. 2019; Miranda and Sousa 2018).

Strengths of this study include clinically confirmed ASD diagnosis by trained psychologists using gold standard diagnostic instruments. Furthermore, our multiple maternal serum samples collected during pregnancy may better represent fetal exposure to PFAS during critical time windows of neurodevelopment than individual samples. However, several limitations should be noted. A relatively small sample size limited the statistical power of our analysis. In addition, as chance findings may not be excluded, further studies should be conducted with a large sample size. There might be residual confounding by unmeasured variables such as lifestyle, behavior, or socioeconomic status because the primary exposure sources of PFAS are diet, drinking water, or dust ingestion (Jian et al. 2017). In this study, we did not include children who did not complete the study for final diagnosis or mothers who did not provide blood samples during pregnancy. As some mothers may have dropped out the study after having girls due to the lower ASD rates in females, there might be potential selection bias in this study. Because MARBLES children have increased genetic susceptibility, null associations for most PFAS in the current study could indicate that genetic factors may have contributed to the development of ASD in this study population more than environmental factors such as exposure to PFAS during pregnancy. Except for two mothers, all children included in the current study had at least one older sibling. Thus, our results should be interpreted with caution and may not extrapolate to the general population such as those children without ASD siblings or who were delivered from first pregnancies. Furthermore, as a growing body of literature suggests environmental factors can interact with genetic factors in the development of ASD (Chaste and Leboyer 2012; Tordjman et al. 2014), further studies using integrated approaches including gene and environment interactions are warranted.

## 5. Conclusions

In the current study that used data from a high-risk ASD cohort, we observed evidence for positive associations between prenatal serum concentrations of PFOA and PFNA and increased risk of child ASD. We also observed that individual as well as combined PFAS showed different effects on ASD risk by advanced maternal age at delivery. In order to confirm these findings, future research is warranted based on general populations without high-risk genetic predisposition and with a larger

sample size.

## 6. Ethics approval and consent to participate

The MARBLES study protocol and this study were approved by the institutional review boards for the State of California, the University of California-Davis (UC-Davis), and the University of Texas-Arlington (UT-Arlington). Participants provided written informed consent before collection of any data. The analysis of coded specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined by CDC not to constitute engagement in human subject research.

## 7. Author's contributions

HS, DB, and IH conceived the study and oversaw its coordination. RS also oversaw its coordination. JO conducted data analyses and drafted the initial manuscript. HS, DB and IH helped oversee the study. AC analyzed PFAS in maternal serum samples. DT advised on data analysis, interpretation, and reporting. DR oversaw assessment of children for ASD. All authors read and approved the final manuscript.

## Declaration of Competing Interest

Rebecca Schmidt has received lodging for the Baby Siblings Research Consortium Meeting; travel and lodging for invited talks at the University of Sherbrooke, Sherbrooke, Québec, Canada; the University of California, Santa Cruz, California (Lodging); Epigenomics 2016, Puerto Rico (Lodging); Neurotoxicity Society & International Neurotoxicology Association, Florianópolis, Brazil; RISE 2017 Second International Meeting on Environmental Health in Strasbourg, France. Rebecca Schmidt also received Autism Speaks grant funding to develop an online autism environmental questionnaire. Other authors declare they have no actual or potential competing financial interests.

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*Disclaimer*

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106328.

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